GLUT-1 AS A RECEPTOR FOR HTLV ENVELOPES AND ITS USES

The invention relates to the use of the ubiquitous vertebrate glucose transporter GLUT1 represented by SEQ ID NO : 2, or of fragments or sequences derived thereof, for the *in vitro* diagnosis of cancers, when used as a tumor marker, or for the screening of compounds useful for the preparation of drugs for the prevention or the treatment of pathologies linked to an infection of an individual with a PTLV, or pathologies linked to an overexpression of GLUT1 on cell surfaces, or the *in vitro* detection of GLUT1 on cell surfaces. The invention also relates to pharmaceutical compositions containing GLUT1, or fragments or sequences derived thereof, and their uses such as in the frame of the prevention or the treatment of pathologies linked to an infection of an individual with a PTLV.

The human T-cell leukemia virus (HTLV) is associated with leukemia and neurological syndromes. The role of viral envelopes in HTLV physiopathology is unclear and the envelope receptor, found in all vertebrate cell lines, remains unidentified.

HTLV envelope glycoproteins induce syncytium formation in vitro but their physiopathological effects are unclear. All vertebrate cell lines express functional HTLV envelope receptors, including cells resistant to HTLV envelope-mediated syncytium formation. We found that expression of the HTLV receptor-binding domain decreased lactate production due to diminished glucose consumption whereas binding-defective envelope mutants did not alter glucose metabolism. Glucose starvation increased HTLV receptor expression, reminiscent of nutrient sensing responses. Accordingly, overexpression of GLUT-1, the ubiquitous vertebrate glucose transporter, specifically increased HTLV envelope binding and GLUT-1 colocalized with HTLV envelopes. Moreover, HTLV envelope binding was highest in human erythrocytes, where GLUT-1 is abundantly expressed and is the sole glucose transporter isoform. These results demonstrate that GLUT-1 is an HTLV envelope receptor, and that this ligand/receptor interaction likely participates in the immunological and neurological disorders associated with HTLV infection.

Thus, the invention relates to the use of the ubiquitous vertebrate glucose transporter GLUT1 represented by SEQ ID NO : 2, or of fragments or sequences derived thereof, said fragments or derived sequences being able to bind to the envelope proteins of the primate T-cell leukemia viruses (PTLV), or of cells expressing GLUT1, for:

- the screening of compounds useful for :

* the preparation of drugs for the prevention or the treatment of pathologies linked to an infection of an individual with a PTLV,

* the preparation of drugs for the prevention or the treatment of pathologies linked to an overexpression of GLUT1 on cell surfaces,

* the *in vitro* detection of GLUT1 on cell surfaces,

said compounds being selected for their ability to bind specifically to said GLUT1,

- the detection, concentration, and/or purification of PTLV or variants thereof, or of PTLV envelope proteins, or fragments thereof,

- the preparation of drugs for the prevention or the treatment of pathologies either linked to an infection of an individual or an animal with a PTLV, such as HTLV-1, HTLV-2, STLV-1, STLV-2, STLV-3, or their variants, or linked to the presence of PTLV SU-related sequences in such individuals or animals,

- the *in vitro* diagnosis of cancers, when used as a tumor marker.

For illustration purpose, screened compounds mentioned above can be selected for their ability to bind specifically to said GLUT1, or fragments of GLUT1, according to the following method using a EGFP-tagged GLUT1-binding component derived from PTLV RBD (receptor binding domain) as an example of such compound able to bind to GLUT1.

A EGFP-tagged Glut1-binding component derived from PTLV RBD is applied onto live or fixed suspension or attached cells. After washes with appropriate buffer, cells are incubated for 30 min at RT, washed and analyzed or quantified as attached on an appropriate support on a fluorescent microscope or as individual cell suspension on a fluorescent analysis ell sorter (FACS). Alternatively, a non-fluorescent GLUT1-binding component derived from PTLV RBD is applied as described above and revealed with a secondary fluorochrome-tagged reagent such as a fluorochrome-tagged secondary antibody directed against the PTLV RBD or against a non fluorochrome tag attached to the said PTLV RBD component.

The invention relates more particularly to the use as defined above, of fragments of GLUT1 chosen among the followings :

- SEQ ID NO : 25 : NAPQKVIEEFY
- SEQ ID NO : 26 : NQTWVHRYGESILPTTLTTLWS
- SEQ ID NO : 27 : KSFEMLILGR
- SEQ ID NO : 28 : DSIMGNKDL
- SEQ ID NO : 29 : YSTSIFEKAGVQQP
- SEQ ID NO : 30 : EQLPWMSYLS

- SEQ ID NO : 31 : QYVEQLC
- SEQ ID NO : 32 : IVGMCFQYVEQLC

These fragments of GLUT1 correspond to the predicted extracellular loops of human GLUT1 as described by Mueckler, M., and C. Makepeace. 1997. Identification of an amino acid residue that lies between the exofacial vestibule and exofacial substrate-binding site of the GLUT1 sugar permeation pathway. J Biol Chem. 272(48):30141-6.

The invention also concerns the use of compounds selected for their ability to bind specifically to GLUT1 as defined above, for the preparation of drugs for the prevention or the treatment of pathologies linked to an infection of an individual with a PTLV, such as pathologies corresponding to adult T cell leukemia (ATL), HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP), as well as other HTLV-associated syndromes such as large granular lymphocyte (LGL) leukaemia (Loughran, T. P., K. G. Hadlock, R. Perzova, T. C. Gentile, Q. Yang, S. K. Foung, and B. J. Poiesz. 1998. Epitope mapping of HTLV envelope seroreactivity in LGL leukaemia. Br J Haematol. 101(2):318-24.), uveitis (Mochizuki, M., A. Ono, E. Ikeda, N. Hikita, T. Watanabe, K. Yamaguchi, K. Sagawa, and K. Ito. 1996. HTLV-I uveitis. J Acquir Immune Defic Syndr Hum Retrovirol. 13 Suppl 1:S50-6.), infective dermatitis (La Grenade, L., R. A. Schwartz, and C. K. Janniger. 1996. Childhood dermatitis in the tropics: with special emphasis on infective dermatitis, a marker for infection with human T-cell leukemia virus-I. Cutis. 58(2):115-8.), arthropathies (Nishioka, K., T. Sumida, and T. Hasunuma. 1996. Human T lymphotropic virus type I in arthropathy and autoimmune disorders. Arthritis Rheum. 39(8):1410-8.), cutaneous T cell lymphoma (mycosis fungoides) (1.Hall, W. W., C. R. Liu, O. Schneewind, H. Takahashi, M. H. Kaplan, G. Roupe, and A. Vahlne. 1991. Deleted HTLV-I provirus in blood and cutaneous lesions of patients with mycosis fungoides. Science. 253(5017):317-20. 2. Zucker-Franklin, D., B. A. Pancake, M. Marmor, and P. M. Legler. 1997. Reexamination of human T cell lymphotropic virus (HTLV-I/II) prevalence. Proc Natl Acad Sci U S A. 94(12):6403-7), polymyositis (Saito M, Higuchi I, Saito A, Izumo S, Usuku K, Bangham CR, Osame M. Molecular analysis of T cell clonotypes in muscle-infiltrating lymphocytes from patients with human T lymphotropic virus type 1 polymyositis. J Infect Dis. 2002 Nov 1;186(9):1231-41), and potentially other idiopathic diseases in which PTLV or PTLV sequences may be involved.

The invention relates more particularly to the use for the preparation of drugs for the prevention or the treatment of pathologies linked to an infection of an individual with a PTLV, of compounds chosen among the followings :

- androgenic steroids (36: May JM, Danzo BJ. Photolabeling of the human erythrocyte glucose carrier with androgenic steroids. Biochim Biophys Acta. 1988 Aug 18;943(2):199-210),

- cytochalasin B, forskolin, dipyridamole, isobutylmethylxanthine (20: Hellwig B, Joost HG. Differentiation of erythrocyte-(GLUT1), liver-(GLUT2), and adipocyte-type (GLUT4) glucose transporters by binding of the inhibitory ligands cytochalasin B, forskolin, dipyridamole, and isobutylmethylxanthine. Mol Pharmacol. 1991 Sep;40(3):383-9),

- ethanol (Krauss SW, Diamond I, Gordon AS. Selective inhibition by ethanol of the type 1 facilitative glucose transporter (GLUT1). Mol Pharmacol. 1994 Jun;45(6):1281-6),

- genistein (Vera JC, Reyes AM, Carcamo JG, Velasquez FV, Rivas CI, Zhang RH, Strobel P, Iribarren R, Scher HI, Slebe JC, et al. Genistein is a natural inhibitor of hexose and dehydroascorbic acid transport through the glucose transporter, GLUT1. J Biol Chem. 1996 Apr 12;271(15):8719-24),

- cadmium (Lachaal M, Liu H, Kim S, Spangler RA, Jung CY. Cadmium increases GLUT1 substrate binding affinity in vitro while reducing its cytochalasin B binding affinity. Biochemistry. 1996 Nov 26;35 (47):14958-62),

- barbiturate (el-Barbary A, Fenstermacher JD, Haspel HC. Barbiturate inhibition of GLUT-1 mediated hexose transport in human erythrocytes exhibits substrate dependence for equilibrium exchange but not unidirectional sugar flux. Biochemistry. 1996 Dec 3;35(48):15222-7),

- dehydroascorbic acid (Rumsey SC, Kwon O, Xu GW, Burant CF, Simpson I, Levine M. Glucose transporter isoforms GLUT1 and GLUT3 transport dehydroascorbic acid. J Biol Chem. 1997 Jul 25;272(30):18982-9),

- tricyclic antidepressants (Pinkofsky HB, Dwyer DS, Bradley RJ. The inhibition of GLUT1 glucose transport and cytochalasin B binding activity by tricyclic antidepressants. Life Sci. 2000;66(3):271-8.),

- oestradiol, genistein and the anti-oestrogens, faslodex (ICI 182780), tamoxifen (Afzal I, Cunningham P, Naftalin RJ. Interactions of ATP, oestradiol, genistein and the anti-oestrogens, faslodex (ICI 182780) and tamoxifen, with the human erythrocyte glucose transporter, GLUT1. Biochem J. 2002 Aug 1;365(Pt 3):707-19),

- gamma agonists of peroxisome proliferator-activated receptors (PPAR) such as thiazolidinedione (troglitazone, pioglitazone, ro siglitazone) ("TZDs modify astrocyte metabolism and mitochondrial function, which could be beneficial in neurological conditions where glucose availability is reduced" from Dello Russo C, Gavrilyuk V, Weinberg G,

Almeida A, Bolanos JP, Palmer J, Pelligrino D, Galea E, Feinstein DL.. Peroxisome proliferator-activated receptor gamma thiazolidinedione agonists increase glucose metabolism in astrocytes. J Biol Chem. 2003 Feb 21;278(8):5828-36).

The invention also relates to the use of compounds selected for their ability to bind specifically to GLUT1 as defined above, for the preparation of drugs for the prevention or the treatment of pathologies linked to an overexpression of GLUT1 on cell surfaces, such as :

- cancers, such as :

. squamous cell carcinoma (Kunkel M, Reichert TE, Benz P, Lehr HA, Jeong JH, Wieand S, Bartenstein P, Wagner W, Whiteside TL. Cancer. 2003 Feb 15;97(4):1015-24),

. hypopharyngeal carcinoma (Mineta H, Miura K, Takebayashi S, Misawa K, Araki K, Misawa Y, Ueda Y. Anticancer Res. 2002 Nov-Dec;22(6B):3489-94),

. breast cancer (Brown RS, Wahl RL.Overexpression of Glut-1 glucose transporter in human breast cancer. An immunohistochemical study. Cancer. 1993 Nov 15;72(10):2979-85),

. cervical carinoma (Mendez LE, Manci N, Cantuaria G, Gomez-Marin O, Penalver M, Braunschweiger P, Nadji M. Expression of glucose transporter-1 in cervical cancer and its precursors. Gynecol Oncol. 2002 Aug;86(2):138-43),

. ovarian carcinoma (Cantuaria G, Fagotti A, Ferrandina G, Magalhaes A, Nadji M, Angioli R, Penalver M, Mancuso S, Scambia G.GLUT-1 expression in ovarian carcinoma: association with survival and response to chemotherapy. Cancer. 2001 Sep 1;92(5):1144-50),

. lung cancer (Ito T, Noguchi Y, Satoh S, Hayashi H, Inayama Y, Kitamura H. Expression of facilitative glucose transporter isoforms in lung carcinomas: its relation to histologic type, differentiation grade, and tumor stage. Mod Pathol. 1998 May;11(5):437-43. Younes M, Brown RW, Stephenson M, Gondo M, Cagle PT. Overexpression of Glut1 and Glut3 in stage I nonsmall cell lung carcinoma is associated with poor survival. Cancer. 1997 Sep 15;80(6):1046-51),

. pancreatic cancer (Reske SN, Grillenberger KG, Glatting G, Port M, Hildebrandt M, Gansauge F, Beger HG. Overexpression of glucose transporter 1 and increased FDG uptake in pancreatic carcinoma. J Nucl Med. 1997 Sep;38(9):1344-8),

. insulinoma (1: Boden G, Murer E, Mozzoli M. Glucose transporter proteins in human insulinoma. Ann Intern Med. 1994 Jul 15;121(2):109-12,

- inflammatory conditions,

- immune or auto-immune diseases, such as :

. autoimmune myocarditis (Tokita N, Hasegawa S, Tsujimura E, Yutani K, Izumi T, Nishimura T. Serial changes in 14C-deoxyglucose and 201Tl uptake in autoimmune myocarditis in rats. J Nucl Med. 2001 Feb;42(2):285-91),,

. in the frame of CD28 T-cell activation (Frauwirth KA, Riley JL, Harris MH, Parry RV, Rathmell JC, Plas DR, Elstrom RL, June CH, Thompson CB. The CD28 signaling pathway regulates glucose metabolism. Immunity. 2002 Jun;16(6):769-77),

. in the frame of immunomodulation (Moriguchi S, Kato M, Sakai K, Yamamoto S, Shimizu E. Decreased mitogen response of splenic lymphocytes in obese Zucker rats is associated with the decreased expression of glucose transporter 1 (GLUT-1). Am J Clin Nutr. 1998 Jun;67(6):1124-9),

- disorders of the central nervous system, such as facilitated glucose transporter protein type 1 (GLUT1) deficiency syndrome (review in Klepper J, Voit T. Eur J Pediatr. 2002 Jun;161(6):295-304.)

The invention relates more particularly to the use for the preparation of drugs for the prevention or the treatment of pathologies linked to an overexpression of GLUT1 on cell surfaces, of compounds chosen among the followings :

- polypeptides compounds corresponding to the envelope proteins of PTLV, or fragments or sequences derived thereof, said fragments or derived sequences being able to bind to GLUT1,

- glucose or derivatives such as galactose, 2-fluorodeoxyglucose, 2-deoxyglucose, 3-O-methylglucose

- androgenic steroids, cytochalasin B, forskolin, dipyridamole, isobutylmethylxanthine, ethanol, genistein, cadmium, barbiturate, dehydroascorbic acid, tricyclic antidepressants, oestradiol, anti-oestrogens, faslodex (ICI 182780), tamoxifen, gamma agonists of peroxisome proliferator-activated receptors (PPAR) such as thiazolidinedione, troglitazone, pioglitazone, ro siglitazone, as mentioned above.

The invention relates more particularly to the use of polypeptides corresponding to the envelope proteins of PTLV, or fragments or sequences derived thereof, said polypeptides being selected for their ability to bind specifically to the ubiquitous vertebrate glucose transporter GLUT1 represented by SEQ ID NO : 2, or of nucleotide sequences encoding said polypeptides, for the preparation of drugs for the prevention or the treatment of pathologies linked to an overexpression of GLUT1 on cell surfaces, and the *in vitro* diagnosis of said pathologies.

The invention concerns more particularly the use as defined above, of polypeptides able to bind to at least one of the above mentioned fragments of GLUT1 corresponding to SEQ ID NO : 25, SEQ ID NO : 26, SEQ ID NO : 27, SEQ ID NO : 28, SEQ ID NO : 29, SEQ ID NO: 30, SEQ ID NO : 31, and SEQ ID NO : 32.

The invention concerns more particularly the use as defined above, of polypeptides able to bind to at least the fragment of GLUT1 corresponding to SEQ ID NO : 32.

The invention concerns more particularly the use as defined above, of GLUT1 binding polypeptides mentioned above chosen among the followings :

- the envelope protein of HTLV-1 corresponding to SEQ ID NO : 4, or of HTLV-2 corresponding to SEQ ID NO : 6, or of STLV-1 corresponding to SEQ ID NO : 8, or of STLV-2 corresponding to SEQ ID NO : 10, or of STLV-3 corresponding to SEQ ID NO : 12,
- fragments of the envelope proteins of PTLV, said fragments corresponding to polypeptides delimited in their N-terminal extremity by the amino acid located in position 1 to 90, or in position 75 to 90, and in their C-terminal extremity by the amino acid located in position 135 to 245, or in position 135 to 150, of said envelope proteins of PTLV, such as SEQ ID NO : 4, 6, 8, 10, 12,
- fragments of the envelope proteins of PTLV, said fragments corresponding to the following polypeptides :

* the polypeptide delimited in its N-terminal extremity by the amino acid located in position 83 to 89, and in its C-terminal extremity by the amino acid located in position 139 to 145, of the envelope protein of the strain MT-2 of HTLV-1 corresponding to SEQ ID NO : 4,

* the polypeptide delimited in its N-terminal extremity by the amino acid located in position 79 to 85, and in its C-terminal extremity by the amino acid located in position 135 to 141, of the envelope protein of the strain NRA of HTLV-2 corresponding to SEQ ID NO : 6,

* the polypeptide delimited in its N-terminal extremity by the amino acid located in position 83 to 89, and in its C-terminal extremity by the amino acid located in position 139 to 145, of the envelope protein of STLV-1 corresponding to SEQ ID NO : 8,

* the polypeptide delimited in its N-terminal extremity by the amino acid located in position 79 to 85, and in its C-terminal extremity by the amino acid located in position 135 to 141, of the envelope protein of STLV-2 corresponding to SEQ ID NO : 10,

* the polypeptide delimited in its N-terminal extremity by the amino acid located in position 82 to 88, and in its C-terminal extremity by the amino acid located in position 138 to 144, of the envelope protein of STLV-3 corresponding to SEQ ID NO : 12,

* the polypeptide corresponding to the envelope protein of a variant of HTLV-1, said polypeptide having the following sequence SEQ ID NO : 14,

I K K P N P N G G G Y Y L A S Y S D
P C S L K C P Y L G C Q S W T C P Y
T G A V S S P Y W K F Q Q D V

* the polypeptide corresponding to the envelope protein of a variant of HTLV-1, said polypeptide having the following sequence SEQ ID NO : 16,

V K K P N R N G G G Y Y L A S Y S D
P C S L K C P Y L G C Q S W T C P Y
T G A V S S P Y W K F Q Q D V

* the polypeptide corresponding to the envelope protein of a variant of HTLV-1, said polypeptide having the following sequence SEQ ID NO : 18,

I K K P N R N G G G Y Y L A S Y S D
P C S L K C P Y L G C Q S W T C P Y
T G A V S S P Y W K F Q Q D V

* the polypeptide corresponding to the envelope protein of a variant of HTLV-1, said polypeptide having the following sequence SEQ ID NO : 20,

I K K P N R N G G G Y Y L A S Y S D
P C S L K C P Y L G C Q S W T C P Y
T G P V S S P Y W K F Q Q D V

* the polypeptide corresponding to the envelope protein of a variant of HTLV-1, said polypeptide having the following sequence SEQ ID NO : 22,

I K K P N R N G G G Y H S A S Y S D P
C S L K C P Y L G C Q S W T C P Y A G
A V S S P Y W K F Q Q D V N F T Q E V

* the polypeptide corresponding to the envelope protein of a variant of HTLV-2, said polypeptide having the following sequence SEQ ID NO : 24,

I R K P N R Q G L G Y Y S P S Y N D
P C S L Q C P Y L G S Q S W T C P Y
T A P V S T P S W N F H S D V

The invention concerns more particularly the use mentioned above of GLUT1 binding polypeptides as defined above, characterized in that the treated or detected pathologies are the followings :

- solid tumors, such as brain tumors, squamous cell carcinoma, hypopharyngeal carcinoma, breast cancer, cervical carcinoma, ovarian carcinoma, pancreatic cancer, insulinoma,

- inflammatory conditions, such as multiple sclerosis, rhumatoid arthritis,

- immune or auto-immune diseases, such as autoimmune myocarditis, or in the frame of CD28 T-cell activation, or in the frame of immunomodulation, or systemic lupus erythematous,

- disorders of the central nervous system, such as facilitated glucose transporter protein type 1 (GLUT1) deficiency syndrome.

The invention relates more particularly to the use of compounds selected for their ability to bind specifically to GLUT1 as mentioned above, and more particularly GLUT1 binding polypeptides as defined above, for the *in vitro* detection of GLUT1 on cell surfaces in the frame of processes for the *in vitro* diagnosis of pathologies linked to an overexpression of GLUT1 on cell surfaces, such as pathologies defined above, said processes comprising the following steps :

- contacting a biological sample (such as tumor biopsies or cells or tissue manifesting or with a suspected aberrant GLUT1 expression profile) from an individual with a compound, and more particularly a GLUT1 binding polypeptide, as defined above, said compound, or GLUT1 binding polypeptide, being optionally labeled, or susceptible to be recognized by a labeled molecule,

- determining the level of said compound, or GLUT1 binding polypeptide, bound to the cells contained in the biological sample and comparison with the level of binding of said compound, or GLUT1 binding polypeptide, to cells contained in the biological sample from an healthy individual.

The invention concerns more particularly the use of compounds as defined above for the *in vitro* diagnosis of cancers, characterized in that the compounds used are chosen among the compounds defined above selected for their ability to bind specifically to GLUT1.

The invention relates more particularly to the use as defined above, of GLUT1 binding polypeptides, or of nucleotide sequences encoding said polypeptides, for the preparation of drug vectors containing at their surface said polypeptides, said vectors being useful for targeting GLUT1 overexpressing cells for the prevention or the treatment of pathologies linked to an overexpression of GLUT1 on cell surfaces, said vectors containing molecules active against said pathologies, or containing genes in the frame of gene therapy of these pathologies.

The invention relates more particularly to the use as defined above, of GLUT1 binding polypeptides, or of nucleotide sequences encoding said polypeptides, for the preparation of drug vectors containing at their surface GLUT1 binding polypeptides, said vectors being useful for targeting GLUT1 overexpressing tumor cells, or cells involved in the inflammatory mechanism, or activated cells of the immune system, or cells of the central nervous system, for the prevention or the treatment of related pathologies as defined above.

The invention concerns more particularly the use of GLUT1 binding polypeptides, or of nucleotide sequences encoding said polypeptides, for the preparation of drug vectors as defined above, wherein the molecules active against the pathologies are antitumor molecules, or molecules against inflammatory conditions, immune or auto-immune diseases, or disorders of the central nervous system.

The invention also relates to the use of nucleotide sequences encoding polypeptides compounds selected for their ability to bind specifically to GLUT1 as defined above, such as nucleotide sequences encoding the polypeptides defined above, or fragments thereof, for the preparation, by substitution of one or several nucleotides of said nucleotide sequences, of mutant nucleotide sequences encoding corresponding mutant polypeptides unable to bind to GLUT1.

The invention also concerns the use of mutant polypeptides unable to bind to GLUT1 as defined above :

- as a negative control in the frame of the screening of compounds able to bind specifically to the non mutated corresponding polypeptides, and thus liable to be used in the frame of the preparation of drugs for the prevention or the treatment of pathologies linked to an infection of an individual with a PTLV,
- for the preparation of drugs for the prevention or the treatment of pathologies linked to an infection of an individual with a PTLV.

The invention relates more particularly to the use as defined above, of mutant polypeptides corresponding to the polypeptides defined above, wherein :

- D in position 106 and/or Y in position 114 of the envelope protein of HTLV-1 corresponding to SEQ ID NO : 4,
- D in position 102 and/or Y in position 110 or of HTLV-2 corresponding to SEQ ID NO : 6,
- D in position 106 and/or Y in position 114 or of STLV-1 corresponding to SEQ ID NO : 8,

- D in position 102 and/or Y in position 110 or of STLV-2 corresponding to SEQ ID NO : 10,

- D in position 105 and/or Y in position 113 or of STLV-3 corresponding to SEQ ID NO : 12,

- D in position 18 and/or Y in position 26 of the polypeptides corresponding to SEQ ID NO : 14, 16, 18, 20, 22, and 24,

are substituted by another aminoacid, natural or not, such as mutant polypeptides corresponding to the polypeptides mentioned above wherein said D and/or A residues are substituted by A.

The invention also relates to the use of mutant nucleotide sequences encoding corresponding mutant polypeptides unable to bind to GLUT1 as defined above, for the preparation of transgenic mammal cells expressing said mutant polypeptides, said cells having a negative transdominant effect with regard to PTLV, thus preventing infection and dissemination of this latter in the organism.

The invention also concerns pharmaceutical compositions containing GLUT1 represented by SEQ ID NO : 2, or fragments or sequences derived thereof, said fragments or derived sequences being able to bind to the envelope proteins of the primate T-cell leukemia viruses (PTLV), in association with a pharmaceutically acceptable carrier.

The invention relates more particularly to pharmaceutical compositions containing mutant polypeptides corresponding to the polypeptides defined above, wherein :

- D in position 106 and/or Y in position 114 of the envelope protein of HTLV-1 corresponding to SEQ ID NO : 4,

- D in position 102 and/or Y in position 110 or of HTLV-2 corresponding to SEQ ID NO : 6,

- D in position 105 and/or Y in position 113 or of STLV-3 corresponding to SEQ ID NO : 12,

- D in position 18 and/or Y in position 26, of the polypeptides corresponding to SEQ ID NO : 14, 16, 18, 20, 22, and 24,

are substituted by another aminoacid, natural or not, such as mutant polypeptides corresponding to the polypeptides mentioned above wherein said D and/or A residues are substituted by A,

in association with a pharmaceutically acceptable carrier.

The invention also concerns transgenic mammal cells expressing mutant polypeptides unable to bind to GLUT1 as defined above, said cells having a negative transdominant effect

with regard to PTLV, thus preventing infection and dissemination of this latter in the organism.

The invention relates more particularly to pharmaceutical compositions containing transgenic mammal cells as defined above, in association with a pharmaceutically acceptable carrier.

The invention also concerns therapeutic vectors useful for targeting GLUT1 overexpressing cells in pathologies linked to an overexpression of GLUT1 on cell surfaces, such as defined above, said vectors containing at their surface GLUT1 binding polypeptides chosen among those defined above, and containing molecules active against said pathologies, as defined above, or containing genes in the frame of gene therapy.

The invention relates more particularly to pharmaceutical compositions containing therapeutic vectors as described above, in association with a pharmaceutically acceptable carrier.

The invention also relates to a method for the screening of compounds useful for :

* the preparation of drugs for the prevention or the treatment of pathologies linked to an infection of an individual with a PTLV,

* the preparation of drugs for the prevention or the treatment of pathologies linked to an overexpression of GLUT1 on cell surfaces,

* the *in vitro* detection of GLUT1 on cell surfaces,

said method comprising :

- the contacting of GLUT1 represented by SEQ ID NO : 2, or of fragments or sequences derived thereof, said fragments or derived sequences being able to bind to the envelope proteins of the primate T-cell leukemia viruses (PTLV), or of cells expressing GLUT1, with compounds to be tested,

- the selection of compounds able to bind specifically to GLUT1, or fragments or sequences derived thereof, as for example according to the method mentioned above.

The invention relates more particularly to a method for the screening of compounds useful for the prevention or the treatment of pathologies linked to an overexpression of GLUT1 on cell surfaces, and the *in vitro* diagnosis of said pathologies, comprising the steps described above:

The invention also concerns a method for the *in vitro* diagnosis pathologies linked to an overexpression of GLUT1 on cell surfaces, characterized in that it comprises :

- contacting a biological sample (such as biopsies or cells or tissue manifesting or with a suspected aberrant GLUT1 expression profile) from an individual with compounds, and more

particularly polypeptides, selected for their ability to bind specifically to GLUT1 as defined above, said compounds or polypeptides being optionally labeled, or susceptible to be recognized by a labeled molecule,

- determining the level of said compounds or polypeptides bound to the cells contained in the biological sample and comparison with the level of binding of said compound to cells contained in the biological sample from an healthy individual.

The invention relates more particularly to a method as defined above for the *in vitro* diagnosis of pathologies mentioned above.

The invention also concerns a kit for the *in vitro* diagnosis of pathologies linked to an overexpression of GLUT1 on cell surfaces as described above, comprising compounds, and more particularly polypeptides, selected for their ability to bind specifically to GLUT1 as defined above, said compounds or polypeptides being optionally labeled, and, if necessary reagents for the detection of the binding of said compounds or polypeptides to GLUT1 initially present on cell surfaces in the biological sample.

The invention is further illustrated with the detailed description hereafter of the determination of GLUT1 as a specific receptor for PTLV RBD.

The human T-cell leukemia virus (HTLV) type 1 and 2 are present in all areas of the world as endemic or sporadic infectious agents [Slattery, 1999]. The etiological role of HTLV-1 in adult T cell leukemia (ATL) and tropical spastic paraparesis/HTLV-associated myelopathy (TSP/HAM) has been well established [Poiesz, 1980; Yoshida, 1982; Gessain, 1985; Osame, 1986]. The apparently restricted tropism of HTLV to T lymphocytes in infected patients[Cavrois, 1996 ; Hanon, 2000] contrasts with the ability of the viral-encoded envelope glycoprotein (Env) to bind to and direct entry into all vertebrate cell types tested in vitro[Sutton, 1996 ; Trejo, 2000; Kim, 2003]. Retroviral infections depend on early interactions between Env and cellular receptors. Identification of cellular receptors and coreceptors for other retroviral envelopes have helped to elucidate certain aspects of retrovirus physiopathology as well as their transmission and spreading within organisms and populations[Berger, 1999; Clapham, 2001; Weiss, 2002]. However, no clear association between HTLV Env and HTLV-associated diseases has been established and the identity of the receptor(s) for HTLV-1 and HTLV-2 Env has remained elusive.

Numerous cell surface components have been shown to play a role in HTLV Env-mediated syncytia formation [Niyogi, 2001; Daenke, 1999; Hildreth, 1997]. Nevertheless, HTLV Env-dependent cell membrane fusion and syncytia formation appear to be distinct from receptor binding per se [Denesvre, 1996; Daenke, 2000; Kim, 2000; Kim, 2003]. The

search for HTLV Env receptor has been hindered in part by its ubiquitous presence [Sutton, 1996; Trejo, 2000; Jassal, 2001; Kim, 2003]. Additionally, the induction of rampant syncytium formation in cell culture upon expression of HTLV Env [Hoshino, 1983; Nagy, 1983] has prevented efficient and persistent Env expression. Based on our observation that the HTLV Env amino terminal domain shares striking structural and functional homology with that of murine leukemia viruses (MLV), we defined HTLV Env receptor-binding domain (RBD) and derived HTLV Env-based tools that overcome the problem of syncytia formation [Kim, 2000; Kim, 2003]. We were thus able to follow specific interactions between the Env RBD and a primary HTLV receptor. Using these tools, we have previously shown that the HTLV receptor is expressed on the surface on T lymphocytes, the major HTLV reservoir in vivo, only following T cell receptor activation[Manel, 2003].

Here we describe striking metabolic alterations in cell cultures following expression of HTLV envelopes as well as HTLV receptor binding domains. These alterations are characterized by a defect in the acidification of the cell culture medium associated with a decreased lactate production and a decline in glucose consumption and uptake. These observations as well as the knowledge that Env receptors for the related MLV and most of the gammaretrovirus belong to the family of multiple-membrane spanning transporters[Overbaugh, 2001] prompted us to test ubiquitous lactate and glucose transport-associated molecules as receptors for HTLV Env. We show that the ubiquitous GLUT-1 glucose transporter, present in all vertebrates, is an essential and specific component of the receptor for HTLV. Moreover, interaction of GLUT-1 with the entire HTLV-1 and HTLV-2 envelopes as well as the truncated HTLV-1 and HTLV-2 RBDs alters glucose metabolism.

HTLV envelopes alter lactate metabolism

Cell proliferation in standard culture media is accompanied by acidification of the milieu that translates into a color change from red to yellow tones in the presence of the phenol-red pH indicator. Upon transfection of either highly syncytial HTLV-1 and HTLV-2 envelopes, or a non-syncytial chimeric envelope that harbors the HTLV-1 RBD in a MLV Env backbone (H_{183}FEnv), culture medium did not readily acidify, and harbored red tones for several days post-transfection (fig 1a). Moreover, expression of truncated soluble HTLV RBD proteins fused with either GFP, –HA, or –rFc tags also inhibited medium acidification. In contrast, no envelope construct that lacked HTLV RBD, including different MLV group envelopes, feline, porcine, lentiviral and Jaagsiekte retroviral Envs, as well as VSV-G and Ebola glycoproteins, had this effect. The lack of acidification associated with HTLV-1 or

HTLV-2 Env expression was not an indirect consequence of their syncytial activity, since (i) medium acidification was observed in cells expressing a syncytial amphotropic-MLV Env (A-MLV devoid of the R peptide) (fig 1a) and (ii) medium acidification was blocked when HTLV Env was expressed in cells that are resistant to HTLV-Env mediated syncytia formation (NIH3T3 TK$^-$ cells)[Kim, 2003].

Decrease of pH in cell culture is primarily due to extracellular accumulation of lactate [Warburg, 1956]. Lactate is the major byproduct of anaerobic glycolysis *in vitro* and its excretion is mediated by an H+/lactate symporter [Halestrap, 1999]. We monitored lactate content in culture supernatants following transfection of various retroviral envelopes and RBD. Lactate accumulation was consistently 3-fold lower in H_{183}FEnv- and HTLV RBD-transfected cells than in control- or MLV Env-transfected cells (fig 1b). This decrease in extracellular lactate accumulation after HTLV RBD transfection was DNA dose-dependent. Moreover, we found that the decrease in lactate accumulation following transfection of HTLV RBD was apparent as early as 4 hours after the addition of fresh media (fig 1c).

Receptor binding and lactate metabolism

To examine whether a direct relationship exists between binding of the HTLV envelope receptor and diminished extracellular acidification and lactate accumulation, we attempted to generate HTLV-1 RBD ($H1_{RBD}$) mutants with impaired receptor binding capacities. To this end, mutations resulting in single alanine substitutions were introduced at two different positions in $H1_{RBD}$, D106 and Y114 which are highly conserved among primate T-lymphotropic viruses. Although both D106A and Y114A RBD mutants were expressed and secreted as efficiently as the wild-type $H1_{RBD}$ (fig 2a), they exhibited significantly reduced (D106A) or non detectable (Y114A) binding to the HTLV receptor as detected by FACS analysis (fig 2b). Moreover, perturbations in lactate metabolism correlated with binding to the HTLV receptor: lactate accumulation was not reduced in cells expressing the non-binding Y114A RBD mutant and was minimally reduced in cells harboring the D106 RBD (fig 2c). Similar results were obtained with $H2_{RBD}$ harboring the same allelic mutations. These data favor a direct association between lactate-related metabolic alterations and HTLV Env receptor binding.

Extracellular lactate accumulates in cell cultures following its transport across cellular membranes by the MCT1 monocarboxylate transporter[Garcia, 1994]. Because HTLV and MLV share a common organization of the extracellular envelope [Kim, 2000] and the receptors for MLV Env are multispanning metabolite transporters [Overbaugh, 2001], we

assessed whether the HTLV RBD bound to MCT1. Moreover, similar to our previous data concerning expression of the HTLV receptor on T cells [Manel, 2003], expression of MCT1 chaperone CD147 [Kirk, 2000] increases during T cell activation [Kasinrerk, 1992]. However, separate and combined overexpression of MCT1 and CD147 did not result in increased $H1_{RBD}$ binding, arguing against a role for these molecules as receptors for HTLV Env.

HTLV receptor and glucose metabolism

In addition to a decrease in extracellular lactate accumulation, expression of the HTLV RBD also led to decreased intracellular lactate content, indicative of metabolic alterations upstream of lactate transport. In cell cultures, lactate accumulation results from the degradation of glucose during anaerobic glycolysis. Therefore, we assessed whether the decreased accumulation of lactate observed upon expression of HTLV RBD was linked to glucose metabolism. We measured glucose consumption as normalized to cellular protein content. Glucose consumption of cells expressing an HTLV RBD within the context of the H_{183}FEnv entire envelope or the $H1_{RBD}$ was significantly decreased as compared to control cells (fig 3a) and this defect was detectable as early as 8 hours post transfection. To determine if this decrease in glucose consumption corresponded to a decrease in glucose transport across cellular membrane, we measured 2-deoxyglucose and fructose uptake in control cells and cells expressing HTLV RBD (fig 3b). We observed that expression of either HTLV-1 or HTLV-2 RBD induced an approximatively 4-fold decrease in 2-deoxyglucose uptake, while A-MLV RBD had only a minor effect. Inhibitors of glucose uptake, cytochalasin B and phloterin, also inhibited glucose uptake. Theses results were also true for 3-O-methylglucose transport. Fructose uptake in the same cells was not altered by the presence of HTLV-1 nor HTLV-2 RBD however A-MLV RBD induced a slight decreased. We next evaluated the effect of glucose deprivation on the availability of the HTLV receptor in both adherent human 293T cells and suspension Jurkat T cells. After overnight culture of cells in the absence of glucose, binding of $H1_{RBD}$ was consistently increased by 2-fold in both cell types (fig 3c). This effect of glucose deprivation was specific to HTLV as amphotropic MLV RBD (A_{RBD}) binding was only marginally affected (fig 3c). This phenomenon is reminiscent of a general metabolite transport feedback loop, whereby transporter availability at the cell surface increases upon substrate starvation [Martineau, 1972].

HTLV envelopes bind glucose transporter-1

A simple model whereby the HTLV envelope inhibits glucose consumption via direct binding to a glucose transporter can explain the metabolic effects described above. Upon evaluation of the different glucose transporter candidates, GLUT-1 appears to be the only one encompassing all the known properties of the HTLV receptor. Indeed, GLUT-1 expression is increased upon glucose deprivation and is transports glucose in all vertebrate cells [Mueckler, 1985], while fructose is transported by GLUT-5. Furthermore, GLUT-1 is not expressed on resting primary T cells and its expression is induced upon T cell activation [Rathmell, 2000; Chakrabarti, 1994] with kinetics that are strikingly similar to what we have reported for the HTLV receptor [Manel, 2003]. Since human but not murine erythrocytes have been described to be the cells exhibiting the highest concentration of GLUT-1 [Mueckler, 1994], we evaluated HTLV receptor availability on freshly isolated red blood cells. Binding of $H1_{RBD}$ on human erythrocytes was strikingly efficient, reaching levels higher than those observed on any other tested cell type, whereas A_{RBD} binding to erythrocytes was minimal (fig 4a). On murine erythrocytes however, no significant $H1_{RBD}$ binding could be detected, despite a similar A_{RBD} binding on murine and human erythrocytes. Furthermore, primary human hepatocytes do not express GLUT-1. Accordingly, we were unable to detecte $H1_{RBD}$ binding to human primary hepatocytes, while A_{RBD} binding could be readily detected.

In order to directly test the ability of HTLV envelopes to bind GLUT-1, we derived a tagged GLUT-1 expression vector and overexpressed this protein in HeLa cells. Both $H1_{RBD}$ and $H2_{RBD}$ binding was dramatically increased upon GLUT-1 overexpression (fig 4b). This interaction was specific as the HTLV-2 binding-defective mutant, D102A, as well as its HTLV-1 counterpart, D106A, did not bind GLUT-1 (fig 4b). Furthermore, $H1_{RBD}$ and $H2_{RBD}$ binding remained at background levels upon overexpression of the amphotropic MLV envelope receptor, the inorganic phosphate transporter PiT2 [Miller, 1994]. Conversely, binding of A_{RBD} was not increased after GLUT-1 overexpression but as expected, this interaction was increased upon transfection of PiT2 (fig 4b). GLUT-3 is the closest isoform to GLUT-1, and transports glucose with kinetics similar to that of GLUT-1. Thus, we derived a tagged GLUT-3 expression vector. Albeit similar overexpression levels of GLUT-1 and GLUT-3 in 293T cells, GLUT-3 did not induce any increase in $H1_{RBD}$ binding (fig 4c), suggesting that increase $H1_{RBD}$ binding in cells overexpressing GLUT-1 is not an indirect consequence of increased glucose uptake. To determine if GLUT-1 transfected cells were directly responsible for the observed increased in $H1_{RBD}$ binding, we derived fluorescent tagged GLUT-1 and GLUT-3 to uniquevocally identity GLUT-overexpressing cells in the

course of our FACS analysis. In this context, only cells overexpressing GLUT-1-DsRed2 displayed an significant increase in $H1_{RBD}$ binding, while overexpressing GLUT-3-DsRed2 had no effect on $H1_{RBD}$ binding (fig4d). Consequently, we tested if HTLV glycoproteins directly interacts with GLUT-1 proteins. To this end, we evaluated the ability of $H1_{RBD}$ to immunoprecipitate GLUT-1. As shown on fig 4e, GLUT-1 could be readily detected upon immunoprecipitation with anti-rabbit-Fc-beads when it was co-expressed with $H1_{RBD}$, but could not be detected when expressed alone or with the $H1_{RBD}$ Y114A mutant. Moreover, a GFP-tagged HTLV-2 RBD colocalized with GLUT-1 but not with PiT2 as assessed by fluorescence microscopy. Therefore, the GLUT-1 glucose transporter is an essential component of the HTLV envelope receptor.

Interaction of GLUT-1 with its ligand cytochalasin B inhibits glucose transport [Kasahara, 1977]. Since we showed that binding of HTLV envelopes to GLUT-1 inhibits glucose consumption and uptake, we tested whether cytochalasin B would abrogate HTLV RBD binding. Indeed, cytochalasin B treatment of Jurkat T cells dramatically inhibited binding of $H1_{RBD}$, whereas binding of A_{RBD} was not affected (fig 5a). Thus, GLUT-1 directed glucose transport as well as binding of HTLV envelopes to GLUT-1 are similarly inhibited by the cytochalasin B ligand. Altogether, these data demonstrate that GLUT-1 is a receptor for HTLV envelopes.

Viral receptor permits entry and thus infection. No cellular system currently exists that lacks GLUT-1 expression. Thus, we developed a system in which HTLV infection is specifically inhibited at the level of envelope-receptor interaction. In this system, over-expression of HTLV-2 RBD interferes with infecting incoming HTLV particles and specifically decreases HTLV titers by at least 2 logs, while no effect is detected on control A-MLV titers. To determine if GLUT-1 is an entry receptor for HTLV, we overexpressed GLUT-1, GLUT-3 or Pit2 in addition to the interfering $H2_{RBD}$. While Pit2 and GLUT-3 had no effect on HTLV titers, GLUT-1 completely alleviated the interference to infection induced by $H2_{RBD}$ (fig 5b). Interestingly, both GLUT-1 and GLUT-3, but not Pit2, alleviated the alteration of glucose metabolism induced by the HTLV RBD. Thus, GLUT-1 is an entry receptor for HTLV.

Discussion

Here we show that HTLV-1 and -2 envelopes interact with GLUT-1 through their receptor binding domains. This interaction strongly inhibits glucose consumption and glucose uptake, leading to decreased lactate production and a block in extracellular milieu

acidification. Mutations that specifically altered receptor binding of both HTLV-1 and 2 envelopes released the block in glucose consumption, indicative of a direct correlation between receptor binding determinants in the HTLV envelopes and glucose transport. Glucose starvation was rapidly followed by increased binding of HTLV envelopes, highlighting a nutrient-sensing negative feedback loop between glucose availability and cell surface HTLV receptor expression. Further evidence converged to identify GLUT-1 as the receptor, including increased binding of HTLV RBD upon overexpression of GLUT-1 but not GLUT-3, immunoprecipitation of GLUT-1 by $H1_{RBD}$ but not the receptor-binding mutant $H1_{RBD}$ Y114A, uppermost binding of HTLV RBD on human erythrocytes, where GLUT-1 is the major glucose transporter isoform, and no binding of HTLV RBD on human primary hepatocytes and murine erythrocytes, where GLUT-1 is minimally expressed. Finally, GLUT-1 could specifically alleviate interference to infection induced by HTLV RBD. GLUT-1 fits all other known properties of the HTLV receptor. Indeed, as previously demonstrated for the HTLV receptor [Manel, 2003], GLUT-1, but not the GLUT 2-4 isoforms, is not expressed on resting T lymphocytes [Chakrabarti, 1994; Korgun, 2002] and is induced upon immunological [Frauwirth, 2002; Yu, 2003] or pharmacological [Chakrabarti, 1994] activation. Moreover, GLUT-1 orthologues are highly conserved among vertebrates, but are highly divergent between vertebrates and insects [Escher, 1999].

GLUT-1 is thus a new member of the multimembrane spanning metabolite transporters that serve as receptors for retroviral envelopes. Interestingly, until now, all envelopes that recognize these receptors have been encoded by retroviruses that have a so-called simple genetic organization, such as MLV, feline leukemia viruses, porcine endogenous retrovirus and the gibbon ape leukemia virus [Overbaugh, 2001], whereas HTLV belongs to the so-called complex retroviruses which code for several additional regulatory proteins. However, we have shown that in contrast to the wide phylogenetic divergence of their genomic RNA, the envelopes of HTLV and MLV share a similar modular organization with some highly conserved amino acid motifs in their respective receptor binding domains[Kim, 2000].

Cell-to-cell contact appears to be required for HTLV transmission, and the cytoskeleton appears to play a major role in this process [Igakura, 2003]. Indeed, we observed that the HTLV receptor, despite pancellular expression, is specifically concentrated to mobile membrane regions and cell-to-cell contact areas. It should therefore be expected that the HTLV envelope receptor is associated to the cytoskeleton. Importantly, a cytoplasmic-binding partner of GLUT-1, GLUT1CBP, which encodes a PDZ domain, has been reported to link GLUT-1 to the cytoskeleton [Bunn, 1999]. It will therefore be interesting to evaluate the

respective roles of the HTLV envelope, its cytoskeleton-associated cellular partners, such as GLUT-1, GLUT1CBP and their immediate interacting cell components.

Because expression of the HTLV receptor is induced upon glucose starvation, transmission of HTLV may be more efficient in cells that are locally starved for glucose, such as lymphocytes in lymph nodes [Yu, 2003]. Furthermore, the ability of circulating erythrocytes to dock HTLV, as shown here, might provide a means to distribute HTLV to such tissues.

The identification of GLUT-1 as a receptor for HTLV envelopes provides additional clues as to the ubiquitous in vitro expression of the receptor on cell lines and the paradoxical restriction of HTLV tropism to T lymphocytes in vivo. Rapid and dramatic metabolic alterations associated with the blockade of glucose consumption are likely to take place upon expression of the HTLV envelope in vivo, early after infection. Therefore, we propose that in vivo, HTLV infection initially spreads with a large tropism, however early after infection the vast majority of cells that are highly dependent on GLUT-1 activity are rapidly eliminated. In contrast, resting T lymphocytes that have an extremely low metabolic rate and as such are much less dependent on glucose uptake, can tolerate this effect and are therefore maintained in vivo. Furthermore, local imbalances in the access to glucose following HTLV infection may lead to specific physiological alterations [Akaoka, 2001]. In this regard, it will be of interest to study the potential relationship between HTLV-associated neuropathologies and the specific dependence of neurons on GLUT-1 mediated glucose consumption [Siegel, 1998].

Methods.

Cell culture. 293T human embryonic kidney and HeLa cervical carcinoma cells were grown in Dulbecco's modified Eagle medium (DMEM) with high glucose (4.5 g/l) and Jurkat T-cells were grown in RPMI supplemented with 10% fetal bovine serum (FBS) at 37°C in a 5% CO2-95% air atmosphere. For glucose starvation experiments, cells were grown in either glucose-free DMEM (Life Technologies) or glucose-free RPMI - (Dutscher) with 10% dialyzed FBS (Life Technologies) and glucose (1g/l) was supplemented when indicated.

Expression vectors. Full length envelope expression vectors for HTLV-1 (pCEL/2[Denesvre, 1995]) and Friend ecotropic MLV (pCEL/F [Denesvre, 1995]), have been previously described. For the HTLV-2 envelope, a fragment from pHTE2 [Rosenberg, 1998] encompassing the *tax*, *rex* and *env* genes and the 3' LTR was inserted in the pCSI [Battini, 1999] vector (pCSIX.H2). Full length envelope expression vectors for amphotropic MLV

(pCSI.A), or devoid of its R peptide (pCSI.AΔR), and H_{183}FEnv that contains the N-terminal 183 amino acids of the HTLV-1 receptor-binding domain in the F-MLV envelope background, as well as truncated envelope expression vectors, derived from pCSI and encoding either of the first 215 residues of HTLV-1 SU ($H1_{RBD}$), the first 178 residues of HTLV2-SU ($H2_{RBD}$) or the first 397 residues of the amphotropic murine leukemia virus (MLV) SU (A_{RBD}), fused to a C-terminal rabbit IgG Fc tag (rFc) or to EGFP ($H2_{RBD}$-GFP). All point mutations introduced in HTLV-1 and -2 RBD constructs were generated using the quickchange site-directed mutagenesis method and mutations were verified by sequencing. Human *Glut-1* and *Glut*-3 cDNA were amplified by PCR from the pLib HeLa cDNA library (Clontech), and inserted into pCHIX, a modified version of the pCSI vector that contains a cassette comprising a factor Xa cleavage site, two copies of the hemagglutinin (HA) tag, and a histidine tag. The resulting construct (pCHIX.hGLUT1) encodes a GLUT-1 protein with a HA-His tag at the C-terminal end. GLUT-1 and GLUT-3 were also inserted in a modified pCSI vector containing a DsRed2 C-terminal tag. Similarly, human CD147 was amplified from 293T total RNA by RT-PCR and inserted into the pCHIX backbone in frame with the HA-His tag (pCHIX.hCD147).

Envelope expression and metabolic measurements. 293T cells were transfected with the various envelope expression vectors using a modified version of the calcium phosphate method. After an overnight transfection, cells were washed in phosphate-buffered saline (PBS) and fresh medium was added. Media were harvested at the indicated time points, filtered through a 0.45-μm pore-size filter, and lactate and glucose were measured with enzymatic diagnostic kits (Sigma). Values were normalized to cellular protein content using the Bradford assay (Sigma) after solubilization of cells in lysis buffer (50 mM Tris-HCl pH 8.0, 150 mM NaCl, 0.1% sodium dodecyl sulfate, 1.0% Nonidet P-40, 0.5% deoxycholate) and clarification by centrifugation.

Assay of hexose uptake. 2-deoxy-D[1-^{3}H]glucose, D[U-^{14}C]fructose and 3-O-[^{14}C]methyl-D-glucose were obtained from Amersham. Hexose uptake assay were adapted from Harrison et al(REF HARRISON 1991). After transfection, approximatively 250,000 were seeded/well in 24-well plates. The next day, cells were washed two times in PBS, incubated in serum-free DMEM, washed one time in serum-free glucose-free DMEM, and incubated for 20' in 500 μl serum-free glucose-free DMEM modulo inhibitors (20 μM cytochalasin B, 300 μM phloretin; SIGMA). Uptake was initiated by adding labeled hexoses to a final concentration of 0,1 mM (2 μCi/ml for 2-2-deoxy-D[1-^{3}H]glucose and 0,2 μCi/ml for D[U-^{14}C]fructose and 3-O-[^{14}C]methyl-D-glucose) and cells were incubated for 5'

additional minutes. Cells were then resuspended in 500 µl cold serum-free glucose-free DMEM, wash one time in serum-free glucose-free DMEM, and solubilized in 400 µl of 0,1% SDS. 3 µl was used for Bradford normalization, while the rest was used for detection of either ^{3}H or ^{14}C by liquid scintillation in a Beckman counter.

Western blots. Culture media (10 µl) from 293T cells expressing wild type or mutant HTLV-1 RBDs, and/or GLUT-1or GLUT-3 expression vecotor. were subjected to electrophoresis on SDS–15% acrylamide gels, transferred onto nitrocellulose (Protran; Schleicher & Schuell), blocked in PBS containing 5% powdered milk and 0.5% Tween 20, probed with either a 1:5000 dilution of horseradish peroxidase-conjugated anti-rabbit immunoglobulin or 1:2000 dilution of anti-HA 12CA5 (Roche) monoclonal antibody followed by a 1:5000 dilution of horseradish peroxidase-conjugated anti-mouse immunoglobulin, and visualized using an enhanced chemiluminescence kit (Amersham).

Binding assays. Binding assays were carried out as previously described [Manel, 2003]. Briefly, 5 x 10^5 cells (293T, HeLa, Jurkat or freshly isolated human erythrocytes) were incubated with 500 µl of $H1_{RBD}$, $H2_{RBD}$ or A_{RBD} supernatants for 30 min at 37°C, washed with PBA (1% BSA, 0.1% sodium azide in PBS), and incubated with a sheep anti-rabbit IgG antibody conjugated to fluorescein isothiocyanate (Sigma). When indicated, cytochalasin B (20 µM; Sigma) was added to cells for 1 hour prior to binding analyses. Binding was analyzed on a FACSCalibur (Becton Dickinson) and data analysis was performed using CellQuest (Becton Dickinson) and WinMDI (Scripps) softwares.

Infections. 293T cells were transfected in 6-wells plate, and one day after transfection, medium was replaced by high glucose DMEM supplemented with fructose (5 g/l) and non-essential amino acids. The next day, infection was initiated by adding supernatants containing MLV particles pseuodtyped with either HTLV-2 or A-MLV envelopes. The following day, fresh medim was added, and 24 hours later cells were fixed and stained for alkaline phosphatase activity and dark focus of infection were counted. Viral particles were obtained by transfecting 293T cells with pLAPSN, pGagPoule and either pCSIX.H2 or pCSI.A, and harvesting the 0.45µm-filtered supernatants 24 hours latter.

FIGURE LEGENDS

Figure 1 Expression of the HTLV receptor-binding domain alters cellular metabolism. **a**, Medium acidification and syncytia formation in 293T cells one day post-transfection with control DNA or Env expression vectors, including syncytial wild-type HTLV-1 Env and HTLV-2 Env, a non-syncytial chimeric H_{183}FEnv, and syncytial A-MLV ΔR Env. **b**, Extracellular lactate and glucose in the culture medium of 293T cells were measured two days following transfection with an irrelevant DNA (control), F-MLV Env, H_{183}FEnv, HTLV-1 RBD ($H1_{RBD}$) or amphotropic MLV RBD (A_{RBD}) expression vectors. Lactate and glucose concentrations were normalized to cellular protein content. **c**, 2-deoxyglucose and fructose uptake following transfection of 293T with an irrelevant DNA (control), $H1_{RBD}$, $H2_{RBD}$ or A_{RBD} expression vectors. Control cells were also incubated with glucose transporter inhibitors cytochalasin and phloretin. Data are the means of triplicate measures and are representative of two to three independent experiments. **d**, Expression of the HTLV and amphotropic-MLV receptors on 293T (1) and Jurkat T (2) cells cultured overnight in the presence or absence of glucose was monitored by binding of $H1_{RBD}$ and A_{RBD}, respectively.

Figure 2 HTLV recepetor properties correlates with GLUT1 properties. **a**, Expression of the HTLV and amphotropic-MLV receptors at the surface of human and murine erythrocytes, as well as human primary hepatocytes. **b**, $H1_{RBD}$ and A_{RBD} binding to Jurkat cells in the absence or presence of the Glut-1 inhibitor cytochalasin B.

Figure 3 HTLV receptor-binding correlates with altered lactate metabolism. **a**, Expression of $H1_{RBD}$ and the derived mutants D106A and Y114A was monitored by Western blot analysis of the supernatants of 293T cells following transfection with the various expression plasmids. **b**, Binding of $H1_{RBD}$ and the D106A and Y114A mutants to the HTLV receptor on HeLa cells. **c**, Extracellular lactate in the medium of 293T cells one day post transfection with an irrelevant DNA (control), $H1_{RBD}$ or the $H1_{RBD}$ D106A and Y114A mutants. Data are representative of three independent experiments.

Figure 4 GLUT-1 is a receptor for HTLV envelopes. **a**, Binding of $H1_{RBD}$, $H2_{RBD}$, $H2_{RBD}$ D102A mutant, and A_{RBD} to control 293T cells or 293T cells overexpressing either GLUT-1 or PiT2. **b**, Binding of $H2_{RBD}$-EGFP to cells overexpressing GLUT-1-HA or GLUT-

3-HA, and corresponding immuoblots using an anti-HA antibody. c, Immunprecipitation of GLUT-1-HA from 293T cells transfected with either an irrelevant construct, GLUT-1 alone, H1RBD alone, H1RBD Y114A alone, GLUT-1 with $H1_{RBD}$ or GLUT-1 with $H1_{RBD}$ Y114A expression vectors. Immunoprecipitation was performed using anti-rabbit-Fc beads and probed with an anti-HA antibody. Total cell extracts were blotted using an anti-rabbit Fc or an anti-HA antibody.

Figure 5 GLUT-1 is an entry receptor for HTLV. Infections titer of MLV particles pseudotypes with HTLV-2 or A-MLV envelopes on 293T cells following transfection of an irrelevant or interfering $H2_{RBD}$ expression vectors alone or in addition to GLUT-1, GLUT-3 or Pit2 expression vectors.

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